

Figure S1

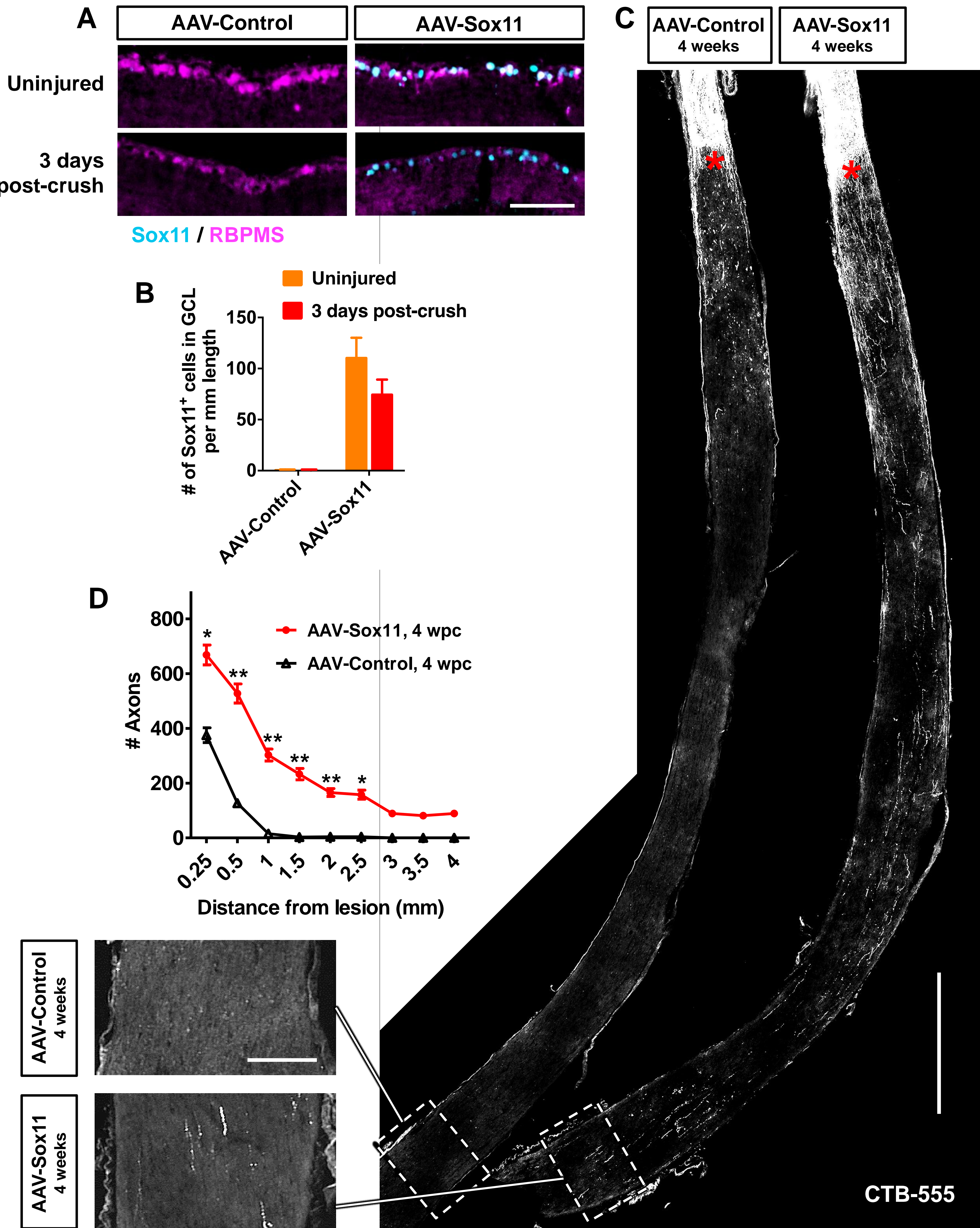


Figure S2

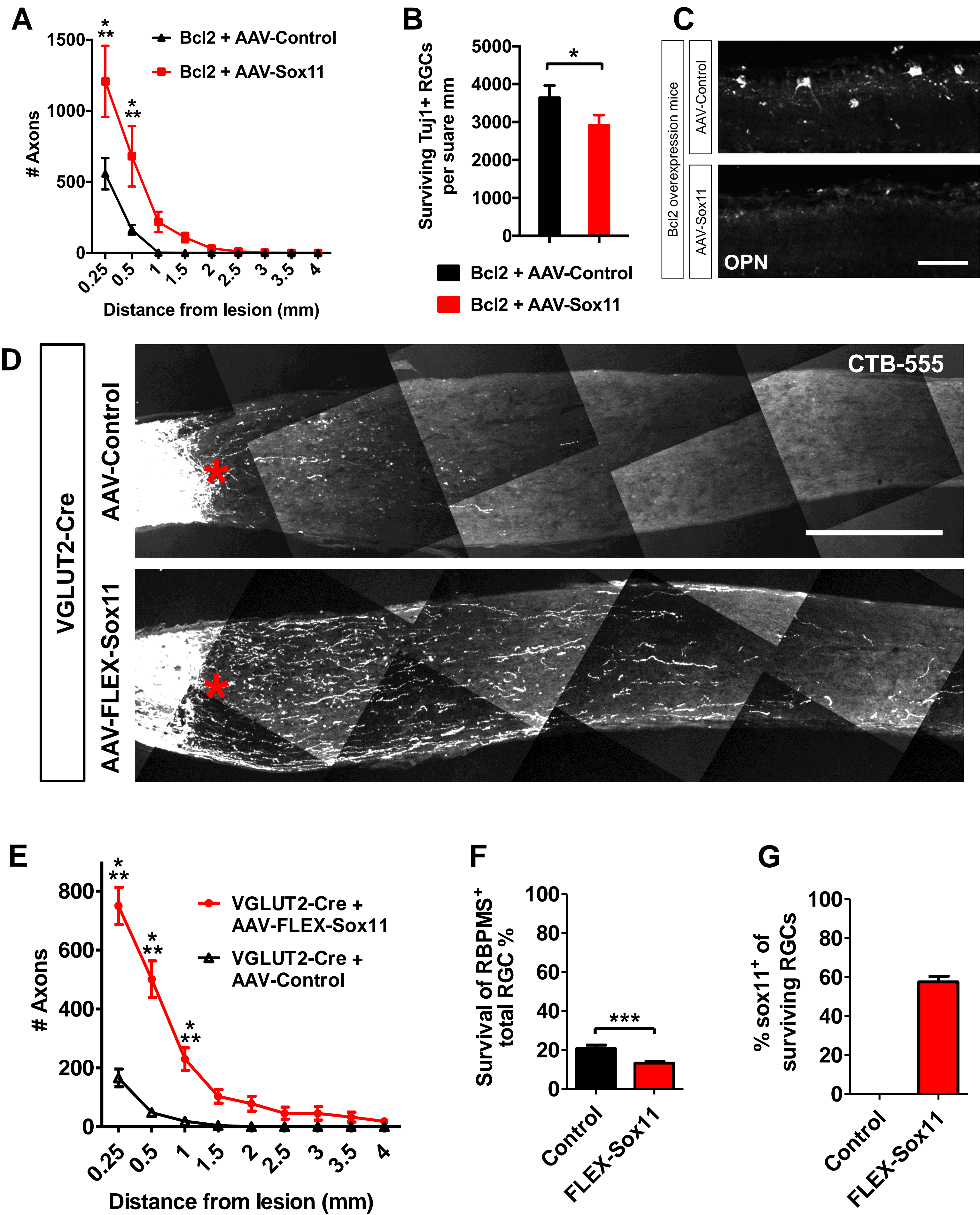


Figure S3

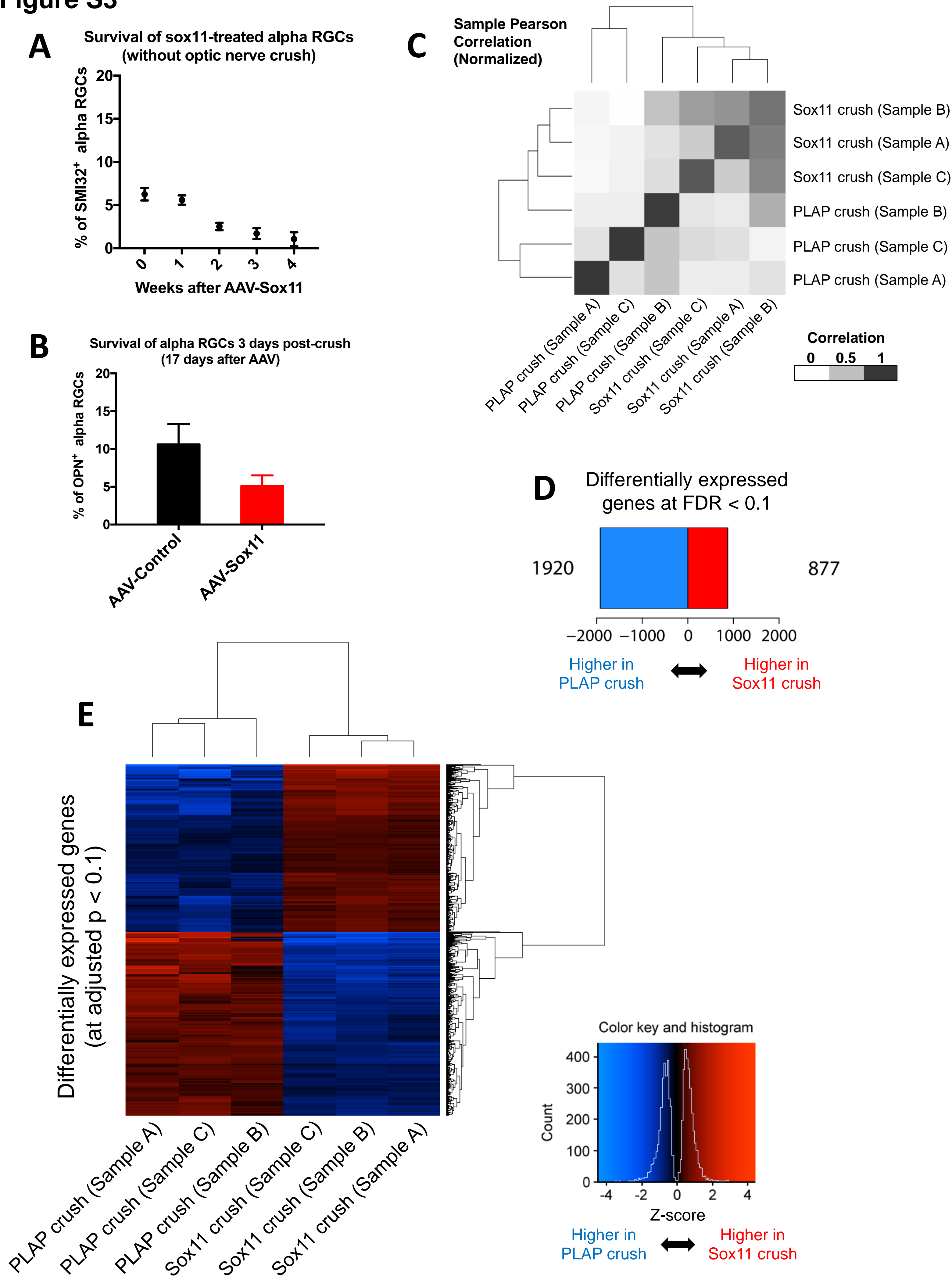


Figure S4

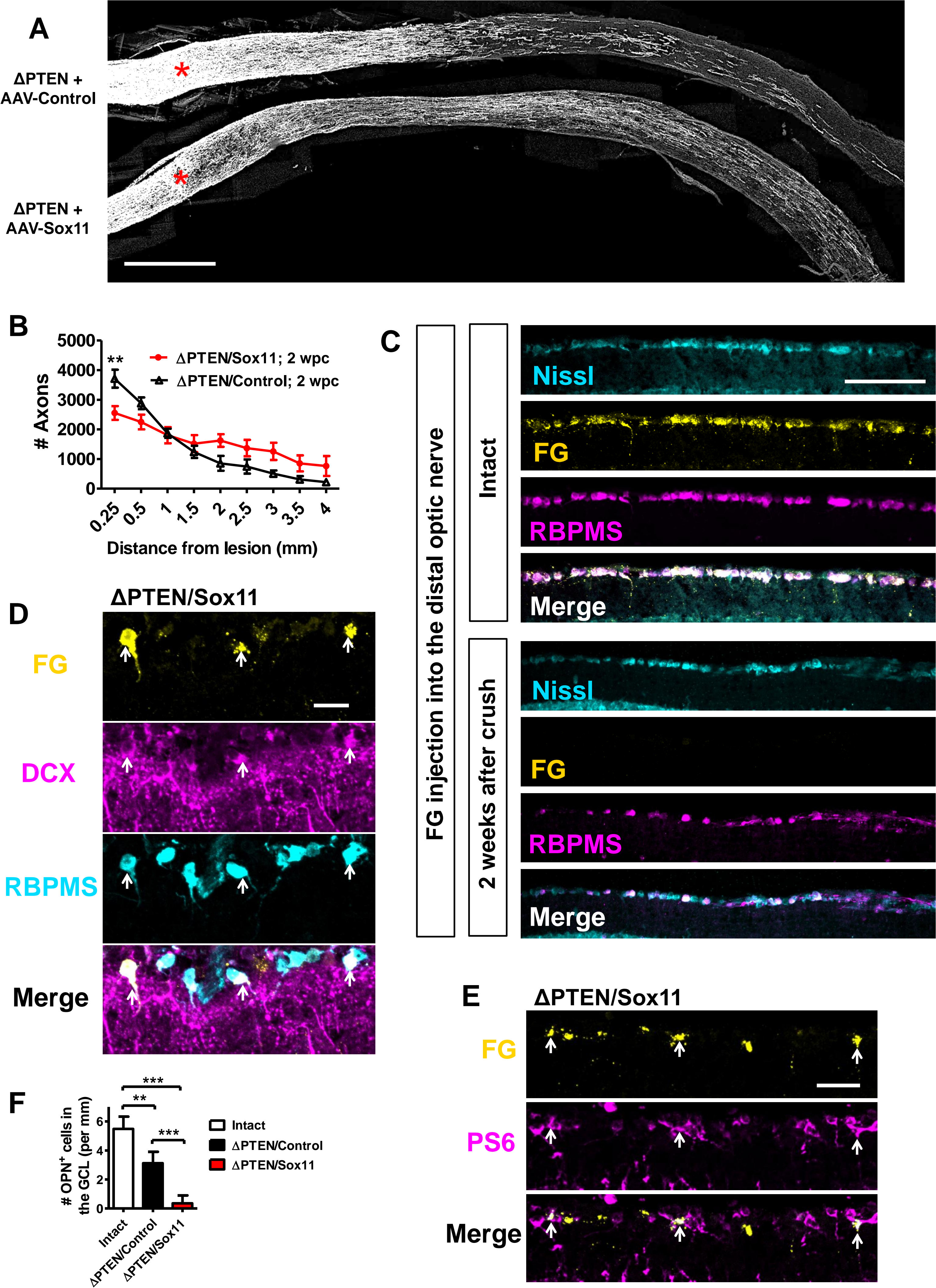


Figure Legends for Supplementary Figures:

Figure S1: Sox11 overexpression greatly exceeds endogenous expression levels, and leads to lengthy, sustained regeneration at 4 weeks after injury, related to Figure 1

(A) Representative retinal sections stained with anti-RBPMS and anti-Sox11 antibodies from retinas injected with AAV-Control (PLAP) or AAV-Sox11, then either kept intact or given optic nerve crush for 3 days before sacrifice. Scale bar represents 50 μ m. **(B)** Quantification of Sox11-positive cells in GCL as in A. **(C)** Representative images of optic nerve sections with CTB-labeled axons from the wild type mice at 4 weeks after injury with prior intravitreal injection of AAV-PLAP or AAV-Sox11. Scale bar represents 500 μ m. **(D)** Quantification of the numbers of regenerating axons in both groups. Data are expressed as mean \pm SEM ($n = 4-6$). * $P < 0.05$, ** $P < 0.01$ (ANOVA with Bonferroni posttests).

Figure S2, Sox11 phenotypes are insensitive to Bcl2 overexpression and are cell autonomous, related to Figures 1 and 2:

(A) Quantification of regenerating axons in mice with Bcl2 overexpression background, injected with either AAV-Control or AAV-Sox11 for 2 weeks, given optic nerve crush, allowed to regenerate for 2 weeks, and given CTB tracer injection before sacrifice. **(B)** Survival of RGCs stained with Tuj1/Tubb3 in flat-mounted retinas in the same experimental conditions as A. **(C)** Retinas in the same experimental conditions as A were sectioned, then stained for Osteopontin. Scalebar represents 50 μ m. **(D)** Vglut2-Cre mice were injected with AAV-Control or AAV-FLEX-Sox11 for 2 weeks, given optic nerve crush, regenerated 2 weeks, and given CTB-555 tracer before sacrifice. Representative sections of optic nerves are shown. Scalebar represents 250 μ m. **(E)** Quantification of regeneration as in D. **(F)** Cell survival of the same experimental conditions as D, quantified from retinas stained with RBPMS. Numbers are relative to RBPMS counts from sections in intact retinas. **(G)** Proportion of RGCs expressing Sox11 from the same conditions as D.

Figure S3: Characterization of the dataset obtained from RNA sequencing for comparing AAV-PLAP to AAV-Sox11 after injury, related to Figure 3.

(A) Timecourse of Alpha RGC elimination from Sox11 expression, without optic nerve crush. Retinal sections from 1, 2, 3, or 4 weeks of Sox11 expression, or in control eyes, were quantified for Smi32+ cells. At 2 weeks of Sox11 overexpression, Alpha RGC ablation is underway but incomplete. We selected this timepoint to include both Alpha RGCs and non-Alpha RGCs in the dataset. **(B)** Mice were injected with AAV-Control or AAV-Sox11 for 2 weeks then sacrificed at 3 days after optic nerve crush. Retinal sections were quantified for Osteopontin+ cells relative to the overall population of RBPMS+ cells in intact retinas. **(C)** Heatmap based on the Pearson correlation matrix between FPKM values for all transcripts of each sample to all other samples analyzed. Dendrograms (at top and at left) indicate relations between samples, as defined by unsupervised clustering. All experimental steps through RNA extraction were performed on three different days for “A”, “B”, and “C” respectively, and all samples with the same letter were performed on the same day. Correlation values are represented in grayscale, with minimum and maximum normalized values of 0 and 1 respectively (key at bottom right). **(D)** Number of differentially expressed genes at the threshold of $FDR < 0.1$. At this threshold, 1920 genes were down-regulated by Sox11, and 877 were up-regulated by Sox11, relative to PLAP control. **(E)** Heat map of differentially expressed genes at the threshold of $p_{\text{adjusted}} < 0.1$. Each row represents one gene’s expression profile. Genes were clustered based on similarity of expression patterns (right dendrogram). Blue to red colors indicate low to high expression respectively, according to the color key (bottom right). Columns indicate individual samples and sample similarity is given by the dendrogram (top).

Figure S4. Combinatorial effect of Sox11 and PTEN deletion at 2 weeks after injury and co-staining of RGCs retrogradely labeled by FluoroGold in intact, non-regenerative and regenerative animals, related to Figure 4.

(A, B) Images (A) and quantification (B) showing CTB-labeled regenerating axons in the optic nerves at 2 weeks post crush (wpc). Scale bar in (A) represents 500 μ m. Data in (B) are expressed as mean \pm SEM ($n = 4-5$). ** $P < 0.01$ (two-way ANOVA with Bonferroni posttests). **(C)** Characterization of a FluoroGold tracing method for retrogradely labeling RGCs with contiguous (intact or regenerating) axons, but not RGCs with injured axons. 4% FG was injected ~ 1.5 mm distal to the common crush site. In wild-type, intact mice, many RGCs are labeled as shown by the location of the FG-positive cells at the retinal ganglion cell layer as well as their co-staining with RGC marker RBPMS. When such FG tracing was performed in wild-type mice 2 weeks after optic nerve crush, no FG labeling was observed at the retinal ganglion cell layer in all mice examined, suggesting FG did not diffuse back to the injury site and was not picked up by axotomized, non-regenerative RGCs. Scale bar represents 100 μ m. **(D)** Co-staining of FG-traced RGCs with DCX and RBPMS in Δ PTEN/Sox11-treated animals 2 weeks after injury. Scale bar represents 20 μ m. **(E)** Co-staining of FG-traced RGCs with the mTOR activity marker phosphor-S6 (PS6) in Δ PTEN/Sox11-treated animals 2 weeks after injury. Scale bar represents 20 μ m. **(F)** Quantification of Osteopontin-expressing alpha RGCs in sections of post-crush retinas with Δ PTEN/Control or Δ PTEN/Sox11 treatments, compared to intact controls. Two sections were analyzed per animal, and numbers indicate the number of positive RGCs per millimeter of ganglion cell layer analyzed. Significance is indicated by **, $P < 0.01$; ***, $P < 0.001$ by ANOVA with Bonferroni posttests ($n = 4-5$ per group).